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New record of the monostiliferous hoplonemertean *Gurjanovella littoralis* Uschakov, 1926 from Japan (Nemertea, Hoplonemertea, Monostilifera)

Natsumi Hookabe[1], Hiroshi Kajihara[2]

1 Graduate School of Science, Hokkaido University, Sapporo, 060-0810, Japan. **2** Faculty of Science, Hokkaido University, Sapporo, 060-0810, Japan.
Corresponding author: Natsumi Hookabe, sofeechan312@gmail.com

Abstract

A single specimen of a monostiliferous hoplonemertean, collected from a depth of 25 m in Akkeshi Bay, northern Japan, represents the first record of *Gurjanovella littoralis* Uschakov, 1926 from the Northwest Pacific. The species has been known only from the type locality, White Sea, Russia, although some planktonic larvae from Oregon, USA, and a juvenile from the Sea of Okhotsk have been identified as a member of the genus by molecular sequence data. Our specimen differs from a topotype from the White Sea by 2.9% of uncorrected *p*-distance and 3.0% of K2P in terms of partial 658-bp sequence of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene. Our COI phylogenetic tree indicates that each of the larvae from Oregon and the Sea of Okhotsk belongs in *Gurjanovella* but represents a different species from *G. littoralis*.

Keywords

DNA barcoding, Hokkaido, marine invertebrates, ribbon worm, Sacconemertidae.

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Introduction

The monostiliferous hoplonemertean *Gurjanovella littoralis* Uschakov, 1926 was originally described based on specimens from the White and the Barents seas, which, respectively, were treated as different subspecies, *Gurjanovella littoralis marisalbi* and *Gurjanovella littoralis murmanica* (Uschakov 1926), and subsequently as varieties *Gurjanovella littoralis* var. *marisalbi* and *Gurjanovella littoralis* var. *murmanica* (Uschakov 1928). Chernyshev (1998) regarded these two taxa as representing different species, and redescribed them as *G. littoralis* and *G. murmanica*, respectively, based on Uschakov's (1926) type material. These two are supposed to be differentiated by the presence (in *G. littoralis*) and absence (in *G. murmanica*) of the ocelli. Chernyshev and Maslakova (2011) provided illustrations of *G. littoralis* based on material from the White Sea. Also, material from the White Sea has been used in Thollesson and Norenburg's (2003) molecular phylogenetic study. Thus far, *G. littoralis* has been only known from the type locality, except for Oregon, USA, from where planktonic larvae have been identified by molecular sequence data (Hiebert and Maslakova unpubl.). The present article reports the occurrence of an adult specimen that is referable to *G. littoralis* for the first time from Japanese waters.

Methods

The single specimen examined in this study was obtained from a silty sediment sample collected by a Smith–McIntire grab sampler in Akkeshi Bay, Hokkaido, Japan, by Dr Keiichi Kakui while onboard the TR/V *Misago-maru* of the Akkeshi Marine Station (AMS). The specimen was anesthetized in a $MgCl_2$ solution isotonic to seawater, photographed alive, and fixed in 99% EtOH. The head was cleared in xylene to observe ocelli. Part of the fixed proboscis was hydrated through an EtOH series, post-fixed in Bouin's fluid for 24 h, dehydrated through an EtOH series, cleared in xylene, and embedded in paraffin. Serial sections of 6–7 µm thickness were stained with Mallory's trichrome method. Voucher material has been deposited in the Invertebrate Collection of the Hokkaido University Museum (ICHUM), Sapporo, Japan.

Total DNA was extracted by a fragment of the ethanol-fixed specimen. A partial sequence of the cytochrome *c* oxidase subunit I (COI) gene was amplified with LCO1490/HCO2198 primer pairs (Folmer et al. 1994) using an Applied Biosystems 2720 thermal cycler. The PCR protocol was as follows: preheating at 94 °C for 2 min; 35 cycles of 94 °C for 40 s, 52 °C for 75 s, and 72 °C for 60 s; then a final extension at 72 °C for 7 min. Nucleotide sequencing was performed using the same primer pairs with an ABI BigDye Terminator ver. 3.1 Cycle Sequencing Kit and an ABI 3100 Avant Genetic Analyzer (Applied Biosystems). The sequence newly obtained in this study has been deposited in the DNA Data Bank of Japan (DDBJ) under accession number LC505450. Uncorrected pairwise genetic distances and Kimura (1980) two-parameter (K2P) genetic distances were calculated based on 658 bp of COI by MEGA ver. 7 (Kumar et al. 2016).

To infer the phylogenetic relationship between the Japanese taxon and its close relatives, the newly obtained COI sequence was combined with six GenBank entries of Sacconemertidae: *Cyanophthalma obscura* (EF208980), *Gurjanovella littoralis* (AJ436904), *Gurjanovella* spp. (KU197600; MF512123), *Sacconemertopsis belogurovi* (KP270884), and Sacconemertidae sp. (KP270883). Alignment for COI was straightforward without indel. GTR + G model was selected as the best-fit partition scheme for maximum-likelihood analysis using PartitionFinder ver. 2.1.1 (Lanfear et al. 2017) employing the greedy algorithm. The maximum-likelihood analysis was carried out with RAxML ver. 8.0.0 (Stamatakis 2014). Nodal values were derived from 1000 bootstrap pseudoreplicates.

Results

Phylum Nemertea
Class Hoplonemertea
Order Monostilifera
Family Sacconemertidae Chernyshev, Timoshkin & Kawakatsu, 1998

Genus *Gurjanovella* Uschakov, 1926

***Gurjanovella littoralis* Uschakov, 1926**

?*Tetrastemma vittata* [sic] Verrill 1874a: 45 (Casco Bay, New England, USA); Verrill 1874b: pl. VII, fig. 3a, b; Verrill 1874c: 389, pl. 2, figs 7, 8 (Casco Bay, New England, USA); Verrill 1879: 185 (Cape Cod Bay, Massachusetts, USA).

?*Tetrastemma vittatum*—Verrill 1892: 411, text fig. 6a, b, pl. XXXV, figs 6, 7 (Long Island Sound, Vineyard Sound, Massachusetts Bay, Casco Bay, Bay of Fundy; Noank; Woods Holl; USA).

?*Cosmocephala cordiceps* Sars in Jensen 1878: 82, pl. VIII, figs 13–16 (Florø, Norway); Levinsen 1879–1880: 201 (Greenland).

?*Chasmocephala cordiceps*—Bergendal 1891: 10 (Greenland).

?*Amphiporus cordiceps*—Friedrich 1933: 496, figs 1–4 (Kiel Bay, Germany); Friedrich 1935: 325, figs 19–21 (Kiel Bay, Germany); Brunberg 1964: 96, text figs 13, 14, pl. 3, figs 5, 7 (Ilefjord, Skinkelmæren, Øresund; Denmark); Băcescu et al. 1971: table 15 (Black Sea, Romania).

Gurjanovella littoralis Uschakov, 1926: 60, pl. II, figs 11, 12 (White Sea, Russia); Uschakov 1928: 413, text figs 6, 7, pl. 4, figs 9–11 (White Sea, Russia); Thollesson and Norenburg 2003: 408 (Kandalaksha Bay, White Sea, Russia); Chernyshev and Maslakova 2011: 18, pl. IX, figs 4–6 (White Sea, Russia); Hiebert and Maslakova unpubl. KU197600 (COI), KU197264 (16S rRNA), KU197265 (16S rRNA) (larvae; Oregon, USA).

New record. JAPAN • 1 specimen, 110 mm in length, 12 mm in maximum width; Hokkaido, Akkeshi Bay; 42°57.625′N, 144°50.961′E; 25 m depth; 21 Jun. 2019; Dr Keiichi Kakui leg.; GenBank: LC505450; ICHUM 5999.

Identification. Anterior fragment of body (lacking posterior end), 110 mm in length, 12 mm in maximum width (Fig. 1A). Body uniformly deep olive-brown, anteriorly more or less elliptical in cross section; dorsoventrally flattened in posterior region. Head demarcated by cephalic furrow encircling neck (Fig. 1B, C); six white bands radiating from the tip, two dorsally, one each laterally, and two ventrally; dorsal ones extending posteriorly behind cephalic furrow. Cephalic furrow paler in color, pointing slightly forward mid-dorsally. Ocelli not evident in living state, but visible in xylene-cleared specimen, 7–8 on each side, arranged in cluster on lateral side near anterior tip of head (Fig. 1D). Proboscis middle chamber with two accessory-stylet pouches, each containing 4–5 accessory stylets. Basis length : width ratio = 2.36 : 1; central stylet : basis length ratio = 0.66 : 1 (Fig. 1E). Proboscis anterior chamber with three muscle layers (outer circular, middle longitudinal, and inner circular muscle layers) and 10 nerves (Fig. 2).

In terms of partial 658-bp COI sequence, genetic distances with respect to *p*-distance/K2P (%) between a topotype from the White Sea (AJ436904), a juvenile from the Sea of Okhotsk (MF512123), and a larva from Oregon, USA (KU197600), were 2.9/3.0, 6.1/6.4, and 5.2/5.5, respectively (Table 1). In the resulting tree, the genus *Gurjanovella* was a sister-taxon to *Cyanophthalma obscura* with a 95% bootstrap value.

Discussion

The six white stripes radiating posteriorly from the cephalic tip, the greenish brown body color, and the

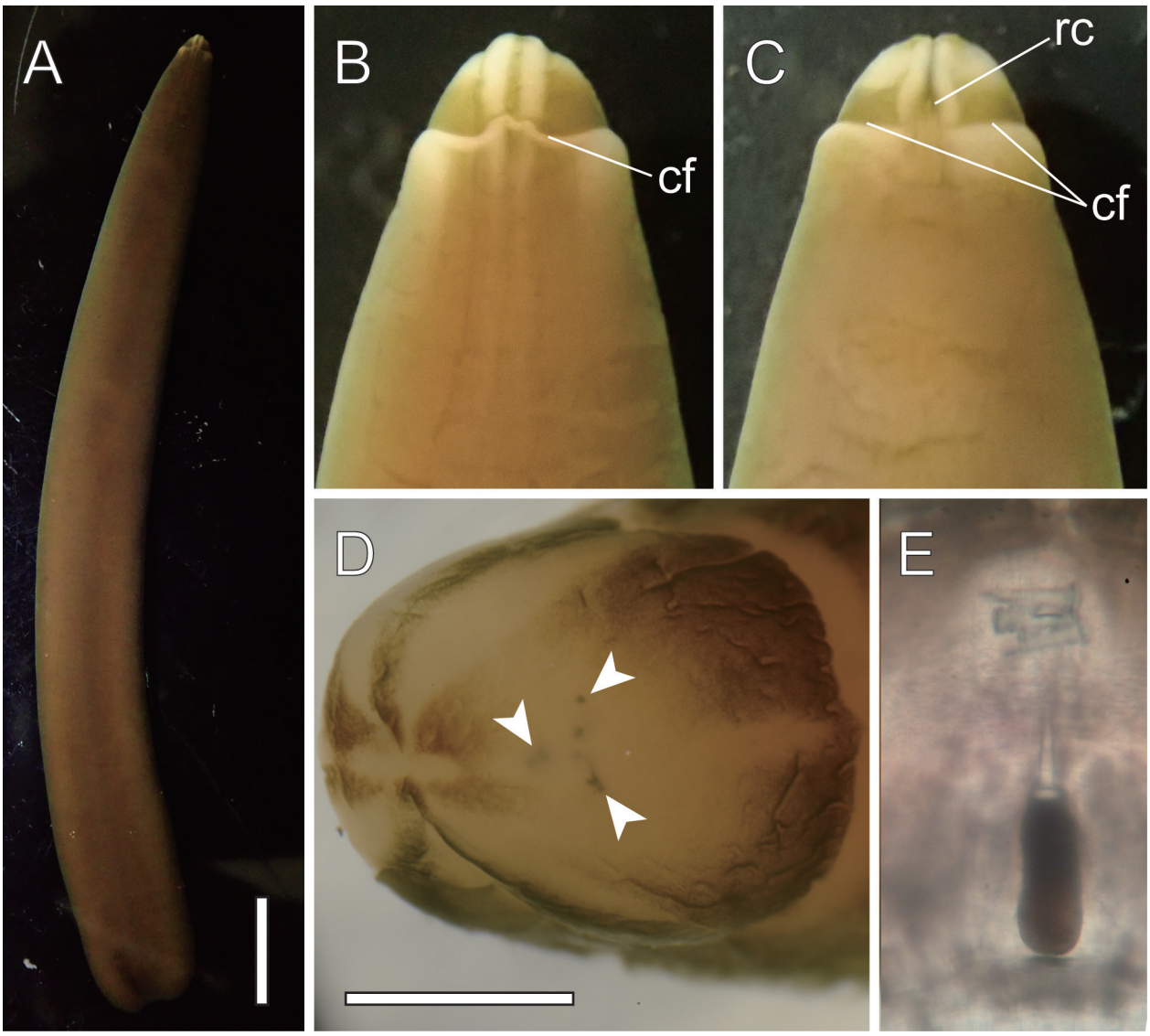

Figure 1. *Gurjanovella littoralis* Uschakov, 1926 from Japan (ICHUM 5999). **A.** General shape of anterior fragment, living state. **B.** Anterior end, dorsal view, living state. **C.** Anterior end, ventral view, living state. **D.** Cephalic tip, anterolateral view, fixed specimen, cleared in xylene; arrowheads indicating ocelli. **E.** Central stylet, basis, and accessory stylets; no scale available. Abbreviations: cf, cephalic furrow; rc, rhynchostome. Scale bars: A = 1 cm; D = 1 mm.

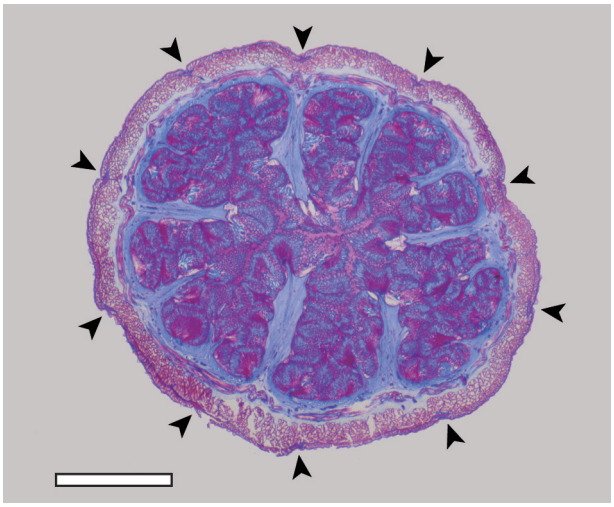

Figure 2. *Gurjanovella littoralis* Uschakov, 1926 from Japan (ICHUM 5999). Photomicrograph of transverse section through proboscis anterior chamber; arrowheads indicating 10 proboscis nerves. Scale bar = 100 µm.

presence of the ocelli in the present specimen are characteristic for *Gurjanovella littoralis* (Ushakov 1926, 1928; Chernyshev and Maslakova 2011). Our Japanese specimen had lost its posterior end when collected. Taking into account another fragment (although eventually not procured) found in the same sediment sample, the intact body length would have well surpassed 15 cm. This is five times greater than the previously known maximum, 3 cm, body length for the species (Ushakov 1926, 1928; Chernyshev and Maslakova 2011). The 2.9–3.0% COI genetic distance between the topotype from the White Sea (Thollesson and Norenburg 2003) and the present Japanese specimen (Table 1) was below 4–10% COI barcoding gap generally observed for the phylum Nemertea (Sundberg et al. 2016), rendering support for our species identification. The larva from Oregon differed by >5.0% from the Russian and Japanese material (Table 1), and thus it might be a different species. In any case, our specimen represents the first record of an adult individual

Table 1. Intraspecific genetic distances (%) between four specimens of *Gurjanovella* Uschakov, 1926 from Akkeshi, Japan (LC505450); the Sea of Okhotsk (MF512123); Oregon, USA (KU197600); and the White Sea, Russia (AJ436904). Values indicate uncorrected *p*-distance (above diagonal) and K2P (below diagonal).

	Akkeshi	Okhotsk	Oregon	White Sea
Akkeshi	—	6.1	5.2	2.9
Okhotsk	6.4	—	5.2	5.2
Oregon	5.5	5.5	—	4.8
White Sea	3.0	5.5	5.0	—

confirmed to be distributed more than 8,000 km away along the coast from the type locality. Given the long swimming juvenile stage of *Gurjanovella* as mentioned in Chernyshev and Polyakova (2018, 2019) and the currently available genetic data, one possibility is that *G. littoralis* consists of genetically diverse individuals with a circum-arctic distribution. Another speculation is that "*Gurjanovella littoralis*" is actually a complex of different cryptic species, especially in view of the Oregonian larvae, along with complicated, potentially synonymous records from northern Europe and the Atlantic coast of the USA (see below). Elucidating the actual species status of *G. littoralis*, as well as its population genetic structure and geographic distribution, should require additional specimens from different localities.

Cosmocephala cordiceps Sars in Jensen, 1878 is undoubtedly a member of *Gurjanovella* (Chernyshev and Maslakova 2011), and possibly even a senior synonym of *G. littoralis*. *Cosmocephala cordiceps* has been recorded from Norway (Jensen 1878) and Greenland (Levinsen 1879; Bergendal 1891); it has also been reported from Germany (Kiel Bay) (Friedrich 1933, 1935), Denmark (Brunberg 1964), and Romania (Black Sea) (Băcescu et al. 1971) as *Amphiporus cordiceps*. *Amphiporus cordiceps* sensu Friedrich (1933) was placed in the genus *Cyanophthalma* Norenburg, 1986 when the latter was established for *Tetrastemma obscurum* Schultze, 1851 (Norenburg 1986). *Cyanophthalma* Norenburg, 1986 is thus to be a junior synonym of *Gurjanovella* Uschakov, 1926 if the two nominal species *G. littoralis* and *T. obscurum* are placed in the same genus; the COI phylogenetic tree (Fig. 3) is indecisive as to this issue. Verrill (1879–1880) mentioned similarity between *C. cordiceps* and *Tetrastemma vittatum* Verrill, 1874, the latter from the Atlantic coast of the USA. Brunberg (1964) also pointed out similarity between the Danish *Amphiporus cordiceps* and *T. vittatum*, which Norenburg (1986) regarded as conspecific, quoting Nathan W. Riser's personal observation.

Confusion exists as to the taxonomy and nomenclature involving *Cosmocephala cordiceps* and related taxa. Gibson (1995) regarded *Cosmocephala cordiceps* sensu Jensen (1878) and *Amphiporus cordiceps* sensu Friedrich (1933) as different taxa, referring to the former as *Amphiporus cordiceps* (Jensen, 1878) and the latter as *Cyanophthalma cordiceps* (Friedrich, 1933). First, Jensen (1878: 82) ascribed *Cosmocephala cordiceps* to M. Sars because the original description of the species was based on Sars' manuscript. Therefore, the author of the specific name should be cited as "Sars in Jensen, 1878" according to Recommendation 51E of the International Code of Zoological Nomenclature (International Commission on Zoological Nomenclature 1999). Second, the author of *Cyanophthalma cordiceps* should be ascribed to Norenburg (1986), instead of Friedrich (1933), if the species is regarded as different from *Cosmocephala cordiceps* Sars in Jensen, 1878 and placed in a different genus. In that case, Norenburg (1986) should be deemed to have established a new taxon by a bibliographic reference to Friedrich (1933), according to Article 13.1.2 of the International Code of Zoological Nomenclature (International Commission on Zoological Nomenclature 1999). Furthermore, if the taxon in question includes *Tetrastemma vittatum* Verrill, 1874 in addition to *Amphiporus cordiceps* sensu Friedrich (1933), but not *Cosmocephala cordiceps* Sars in Jensen, 1878—a concept which would represent the taxonomic interpretation by Norenburg (1986) and Gibson (1995)—then it should be referred to as *Cyanophthalma vittata* (Verrill, 1874), instead of *Cyanophthalma cordiceps* (Friedrich, 1933) as listed in Gibson (1995).

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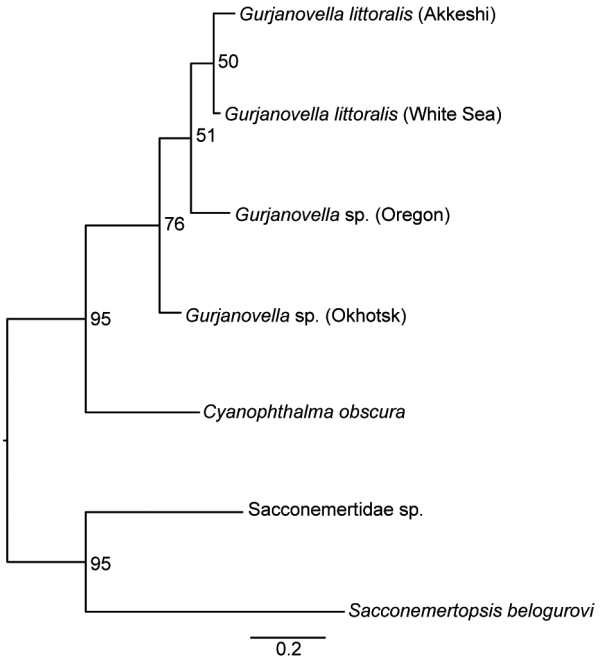

Figure 3. Phylogenetic tree based on COI (658 bp) inferred by a maximum likelihood (ML) analysis, with support values generated by a separate partitioned ML bootstrap analysis based on 1000 replicates.

Authors' Contributions

NH conceived the study, carried out molecular genetics work, and wrote the manuscript. HK photographed and fixed the specimen, prepared the figure, and drafted the manuscript.

References

Băcescu MC, Müller GJ, Gomoiu M-T (1971) Ecologie Marină. Cercetări de ecologie bentală în Marea Neagră; analiza cantitativă, calitativă și comparată a faunei bentale pontice. Vol. 4. Academiei Republicii Socialiste Româna, Bucuresti [Bucharest], 357 pp.

Bergendal D (1891) Kurzer Bericht über eine im Sommer d. J. 1890 unternommene zollogische Reise nach Nord-Grönland. Bihang till Kongl. Svenska Vetenskaps-Akademiens Handlingar 17 (4): 1–20.

Brunberg L (1964) On the nemertean fauna of Danish waters. Ophelia 1: 77–111. https://doi.org/10.1080/00785326.1964.10416273

Chernyshev AV (1998) On the genus *Gurjanovella* Uschakov, 1926 (Nemertea: Enopla). Zoosystematica Rossica 7 (1): 9–11.

Chernyshev AV, Maslakova SA (2011) Phylum Nemertea. In: Sirenko BI (Ed.) Illustrated Keys to free-living invertebrates of Eurasian arctic seas and adjacent deep waters. Vol. 2. Alaska Sea Grant, University of Alaska Fairbanks, Fairbanks, 3–34.

Chernyshev AV, Polyakova, NE (2018) Nemerteans from deep-sea expedition SokhoBio with description of *Uniporus alisae* sp. nov. (Hoplonemertea: Reptantia sl) from the Sea of Okhotsk. Deep Sea Research Part II: Topical Studies in Oceanography 154: 121–139. https://doi.org/10.1016/j.dsr2.2017.09.022

Chernyshev AV, Polyakova NE (2019) Nemerteans from the deep-sea expedition KuramBio II with descriptions of three new hoplonemerteans from the Kuril-Kamchatka Trench. Progress in Oceanography 178: 102148. https://doi.org/10.1016/j.pocean.2019.102148

Chernyshev AV, Timoshkin OA, Kawakatsu M (1998) *Prostoma ohmiense* sp. nov., a new species of freshwater nemertean from Lake Biwa-ko, Central Japan, with special reference to the taxonomy and distribution of the known species in the genus *Prostoma* Dugès, 1828 (Enopla, Hoplonemertea, Monostilifera, Tetrastemmatidae). Bulletin of Fuji Women's College Series II 36: 51–66.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3: 294–299.

Friedrich H (1933) Morphologische Studien an Nemertinen der Kieler Bucht, I und II. Zeitschrift für wissenschaftliche Zoologie 144: 496–509.

Friedrich H (1935) Studien zur Morphologie, Systematik und Ökologie der Nemertinen der Kieler Bucht. Archiv für Naturgeschichte (N.F.) 4: 293–375.

Gibson R (1995) Nemertean genera and species of the world: an annotated checklist of original names and description citations, synonyms, current taxonomic status, habitats and recorded zoogeographic distribution. Journal of Natural History 29: 271–562. https://doi.org/10.1080/00222939500770161

International Commission on Zoological Nomenclature (1999) International Code of Zoological Nomenclature. Fourth edition. The International Trust for Zoological Nomenclature, London, xxiv + 306 pp.

Jensen OS (1878) Turbellarier ved Norges Vestkust. J.W. Eides, Bergen, 97 pp., pls I–VIII.

Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16: 111–120. https://doi.org/10.1007/BF01731581

Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33: 1870–1874. https://doi.org/10.1093/molbev/msw054

Lanfear R, Frandsen PB, Wright AM, Senfeld T, Calcott B (2017) PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. Molecular Biology and Evolution 34: 772–773. https://doi.org/10.1093/molbev/msw260

Levinsen GMR (1879–1880) Bidrag til Kundskab om Grønlands Turbellariefauna. Videnskabelige Meddelelser fra den naturhistoriske Forening i Kjøbenhavn 41–42: 165–204, pl. III.

Norenburg J (1986) Redescription of a brooding nemertine, *Cyanophthalma obscura* (Schultze) gen. et comb. n., with observations on its biology and discussion of the species of *Prostomatella* and related taxa. Zoologica Scripta 15 (4): 275–293. https://doi.org/10.1111/j.1463-6409.1986.tb00229.x

Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30: 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Sundberg P, Kvist S, Strand M (2016) Evaluating the utility of single-locus DNA barcoding for the identification of ribbon worms (phylum Nemertea). PLoS ONE 11 (5): e0155541. https://doi:10.1371/journal.pone.0155541

Thollesson M, Norenburg JL (2003) Ribbon worm relationships: a phylogeny of the phylum Nemertea. Proceedings of the Royal Society B 270: 407–415. https://doi.org/10.1111/j.1096-0031.2011.00376.x

Uschakov P (1926) K faune nemertin Belogo morya. Issledovaniya Russkikh Morei 3: 47–71.

Uschakov ['Uschakow' in print] P (1928) Beschreibung einiger neuen Nemertinenarten vom Barents-Meere, Weißen Meere und Nowaja-Semlja. Zoologische Jahrbücher, Abteilung für Systematik, Ökologie und Geographie der Tiere 54: 407–424.

Verrill AE (1874a) Brief contributions to zoölogy from the Museum of Yale College. No. XXVI. Results of recent dredging expeditions on the coast of New England. No. 4. American Journal of Science and Arts (Series 3) 7: 38–46.

Verrill AE (1874b) Brief contributions to zoölogy from the Museum of Yale College. No. XXIX. Results of recent dredging expeditions on the coast of New England. No. 7. American Journal of Science and Arts (Series 3) 7: 498–505.

Verrill AE (1874c) Explorations of Casco Bay by the U.S. Fish Commission, in 1873. Proceedings of the American Association for the Advancement of Science 22: 340–395.

Verrill AE (1879) Notice of recent additions to the marine Invertebrata, of the northeastern coast of America, with descriptions of new genera and species and critical remarks on others. Part I—Annelida, Gephyraea, Nemertina, Nematoda, Polyzoa, Tunicata, Mollusca, Anthozoa, Echinodermata, Porifera. Proceedings of the United States National Museum 2: 165–205.

Verrill AE (1892) The marine nemerteans of New England and adjacent waters. Transactions of the Connecticut Academy of Arts and Sciences 8: 382–456.